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Insight

Gene pyramids and the balancing act of keeping pests at bay

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Pyramiding *R* genes is a common strategy used by breeders to enhance resistance and increase durability of resistance in crops. However, the molecular mechanisms that mediate *R* gene interactions are not known. Kamphuis *et al.* (2019) analyzed *Medicago truncatula* plants carrying two genes that confer resistance to bluegreen aphids. They identified a potential phytohormone crosstalk triggered by the combined *R* gene action in response to aphid feeding that enhances resistance and minimizes *R* gene-associated fitness costs to the plant.

In the continuous evolutionary struggle to keep up with pest adaptations, plants have acquired resistance (*R*) genes that recognize an invader and eliminate it or reduce its performance. The majority of characterized *R* genes encode nucleotide-binding and leucine-rich receptors, although other types of *R* genes also exist, that recognize pathogen and insect effectors and trigger a defense response (Kourelis and van der Hoorn, 2018). Effector triggered immunity (ETI) mediated by *R* genes can, however, result in fitness costs (Richard *et al.*, 2018).

It is normally accepted that *R* genes trigger a signaling cascade that induces defense responses similar to those triggered by pattern-triggered immunity (PTI), the basal plant immunity response, although *R* gene activation seems to remove the breaks that normally limit the strength of the PTI response, either by boosting the defense response or by removing repressive feedback loops. The resistance response then is stronger, and commonly associated with cell death outcomes (Cui *et al.*, 2015). *R* responses are also normally classified as effective against either biotrophic or necrotrophic pathogens and associated primarily with salicylate or jasmonate signaling, respectively (Spoel and Dong, 2012).

R genes are an important tool for controlling pests and an environmentally friendly alternative to the use of chemical pesticides; although the selective pressure they exert on pests may result in the appearance of pathogens and insects that are indifferent to specific *R* genes. Thus, durability of monogenic resistance traits is a concern (Campbell *et al.*, 2002; Mundt, 2014). A common strategy used by crop breeders to overcome this issue is the use of pyramids of *R* genes that protect against the same pathogen or pest in a single plant line or cultivar (Box. 1). Besides durability of *R* traits, pyramiding also generally results in an increase in resistance when pyramid crops are compared with lines with monogenetic resistance, indicating an additive effect for *R* genes (Mundt, 2018). However, in some cases *R* gene combinations may result in less favorable outcomes suggesting that *R* genes can also have incompatible interactions (Gu *et al.*, 2009).

The *R* gene interactions identified by gene pyramiding also indicate that even when controlling similar output (resistance against a specific pathogen or pest), *R* genes trigger subsets of the plant defense arsenal, and thus combination of *R* genes can result in complementary outcomes and produce more robust responses, or antagonize each other and reduce plant fitness. While these phenomena are well-known from phenotypic analyses associated with breeding programs, the molecular events that mediate *R* gene crosstalk are not known, and few studies have focused on the mechanistic aspect of these interactions.

Kamphuis *et al.* (2019) have now made inroads into this problem. They studied the effect of pyramiding two resistance genes [*AKR* (*Acyrthosiphon kondoi* resistance) and *AIN* (*Acyrthosiphon*-induced necrosis)] that provide resistance against the bluegreen aphid (BGA; *Acyrthosiphon kondoi*) in *Medicago truncatula*. This pyramid results in increased resistance against BGA when compared to plants carrying the individual *R* genes, while at the same time reducing negative growth phenotypes associated with the *AIN* resistance gene following aphid infestation (Fig. 1). *AKR* behaves as a dominant gene and *AIN* is dominant for the presence of necrotic lesions in response to aphids but semi-dominant with respect to direct aphid resistance, measured as decreased aphid performance and reduced effect on aphid-affected damage to the plant. In plants carrying both resistance genes, the effect on aphid resistance seems to be additive; however, *AKR* shows dominant suppression epistasis over the *AIN*-associated necrosis phenotype and plants do not show a negative effect on growth.
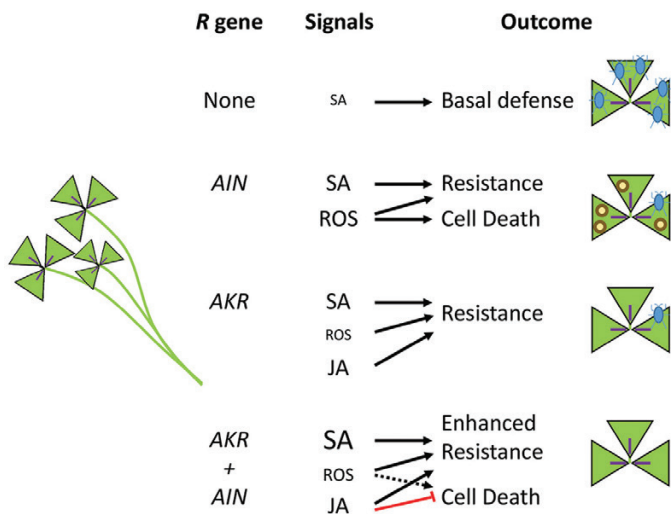
To dissect the molecular mechanisms underlying this complex interaction, Kamphuis and coworkers used a range of approaches, including detection of reactive oxygen species (ROS),

Fig. 1. Pyramiding the *AIN* and *AKR* aphid resistance genes results in a signaling crosstalk that increases resistance while reducing cell death and fitness costs associated with *AIN* (see text).

phytohormone signaling, and aphid feeding behavior by electrical penetration graph (EPG) assays, working with susceptible plants and plants with monogenetic resistance in addition to the resistance pyramid. *Medicago* basal defense response to BGA feeding seems to be controlled by salicylate (SA) signaling, as a sustained SA response is observed in all genotypes. On the other hand, *AKR* induces a unique jasmonate–dependent response to BGA. The *AIN* hypersensitive response (HR) seems to be associated with a large production of ROS and cell death symptoms as manifested by macroscopic necrotic lesions after aphid feeding; while ROS production is also induced by *AKR* but to a lower level, without significant leaf damage. In the pyramid genotype the presence of *AKR* seems to drive the induction of JA signaling while also elevating the SA signal observed in all genotypes. At the same time, although the production of ROS also occurs in the pyramid genotype, it is less

than in plants with *AIN* alone, and the additional presence of *AKR* abolishes the development of large, macroscopic necrotic lesions and instead results in small chlorotic flecks.

How is increased resistance achieved in the pyramid plants? Since the strength of the JA signal appears to be the same in the *AKR-ain* and *AKR-AIN* genotypes, the induction of JA alone does not seem to be the main reason for greater resistance. Thus, it is possible that the combination of *AKR-* and *AIN*–dependent pathways result in an additive effect of the JA and SA–HR pathways to produce the enhanced resistance phenotype. At the same time, the presence of *AKR* suppresses the HR–associated cell death triggered by *AIN*. It is likely that the JA signal induced by *AKR* suppresses the cell death phenotype. In fact, it has previously been shown that JA can attenuate cell death associated with ROS (Xu and Brosché, 2014), or induce antioxidant metabolism that attenuates accumulation of ROS (Yuan *et al.*, 2017). It is interesting that a more subdued ROS production is observed even in lines with *AKR* alone. Thus, it is possible that a highly localized HR still occurs in the *AKR-ain* and *AKR-AIN* genotypes that limits aphid feeding, but the JA-SA and JA-ROS interplay limits the advance of cell death and leaf damage associated with the *AIN* hypersensitive response. From these results it is clear that cell death is not necessary to obtain a robust resistance response to aphids, and that this phenotype is potentially the result of a "runaway" HR and not the cause of resistance (Cui *et al.*, 2015).

Another significant observation from this *R* gene interaction study is the seemingly cooperative interaction between JA and SA signaling. Plants carrying *AKR* + *AIN* have elevated responses for SA when compared to susceptible and monogenetic resistant plants, and they are also more resistant to BGA, while JA recruitment is dependent on the presence of *AKR* but not *AIN*. SA-JA antagonism has been well-studied in Arabidopsis (Robert-Seilaniantz *et al.*, 2011), and this interaction seems to be exploited by hemipterans to colonize Arabidopsis (Walling, 2008). It appears that in legumes,

JA and SA may act synergistically (or at least not antagonistically) to increase resistance to aphids. A similar interaction has been observed in soybean, where pre-treatment with JA increases resistance to soybean aphids, and the increase is not affected by JA+SA co-treatments (Selig *et al.*, 2016). JA and SA signaling also increase simultaneously during the defense responses to soybean aphid feeding observed in susceptible and resistant soybean plants (Li *et al.*, 2008; Studham and MacIntosh, 2013). However, the JA modulation of the SA–HR–cell death pathway suggest that the JA–SA crosstalk is complex and likely occurs at several levels to determine the aphid–resistance outcome observed in these legume–aphid interactions.

While Kamphuis *et al.* (2019) provide a peek at the complex *R* gene interaction that controls resistance to aphids in *Medicago* and their research indicates that phytohormone crosstalk seems to mediate the increase in resistance and fitness observed in the pyramid line, it is clear that much work is still needed to understand the molecular mechanism that facilitates this crosstalk and regulates effective defenses. It will also be important to understand the final products of these defense pathways. Are the changes in aphid feeding observed in EPG experiments the result of chemical defenses or physical barriers associated with the phloem? Is the JA signal induced by AKR directly responsible for these phloem defenses? Given that cell death is not needed for increased resistance, what is the outcome of the ROS and SA signaling pathways? Additional work should also be carried out in other plant–pathogen and plant–insect systems to identify patterns of *R* gene interactions that can provide predictive models for the utilization of gene pyramids in breeding or biotechnology programs.

Keywords: Aphids, gene pyramid, host-plant resistance, *Medicago truncatula*, phytohormone crosstalk, *R* gene.

References

Campbell MA, Fitzgerald HA, Ronald PC. 2002. Engineering pathogen resistance in crop plants. Transgenic Research **11**, 599–613.

Cui H, Tsuda K, Parker JE. 2015. Effector-triggered immunity: from pathogen perception to robust defense. Annual Review of Plant Biology **66**, 487–511.

Djian-Caporalino C, Palloix A, Fazari A, *et al.* 2014. Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. Bmc Plant Biology **14**, 53.

Garza R, Cardona C, Singh SP. 1996. Inheritance of resistance to the bean-pod weevil (*Apion godmani* Wagner) in common beans from Mexico. Theoretical and Applied Genetics **92**, 357–362.

Gu K, Tian D, Qiu C, Yin Z. 2009. Transcription activator-like type III effector AvrXa27 depends on OsTFIIAgamma5 for the activation of Xa27 transcription in rice that triggers disease resistance to *Xanthomonas oryzae* pv. oryzae. Molecular Plant Pathology **10**, 829–835.

Joshi RK, Nayak S. 2010. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. Biotechnology and Molecular Biology Reviews **5**, 51–60.

Kamphuis LG, Klingler JP, Jacques S, Gao L-l, Edwards OR, Singh KB. 2019. Additive and epistatic interactions between *AKR* and *AIN* loci conferring bluegreen aphid resistance and hypersensitivity in *Medicago truncatula*. Journal of Experimental Botany **70**: 4887–4901.

Kourelis J, van der Hoorn RAL. 2018. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. The Plant Cell **30**, 285–299.

Li Y, Zou J, Li M, Bilgin DD, Vodkin LO, Hartman GL, Clough SJ. 2008. Soybean defense responses to the soybean aphid. New Phytologist **179**, 185–195.

McCarville MT, O'Neal ME, Potter BD, Tilmon KJ, Cullen EM, McCornack BP, Tooker JF, Prischmann-Voldseth DA. 2014. One gene versus two: a regional study on the efficacy of single gene versus pyramided resistance for soybean aphid management. Journal of Economic Entomology **107**, 1680–1687.

Mundt CC. 2014. Durable resistance: a key to sustainable management of pathogens and pests. Infection, Genetics and Evolution **27**, 446–455.

Mundt CC. 2018. Pyramiding for resistance durability: theory and practice. Phytopathology **108**, 792–802.

Richard MMS, Gratias A, Meyers BC, Geffroy V. 2018. Molecular mechanisms that limit the costs of NLR-mediated resistance in plants. Molecular Plant Pathology **19**, 2516–2523.

Robert-Seilaniantz A, Grant M, Jones JD. 2011. Hormone crosstalk in plant disease and defense: more than just JASMONATE-SALICYLATE antagonism. Annual Review of Phytopathology **49**, 317–343.

Selig P, Keough S, Nalam VJ, Nachappa P. 2016. Jasmonate-dependent plant defenses mediate soybean thrips and soybean aphid performance on soybean. Arthropod-Plant Interactions **10**, 273–282.

Spoel SH, Dong X. 2012. How do plants achieve immunity? Defence without specialized immune cells. Nature Reviews. Immunology **12**, 89–100.

Studham ME, MacIntosh GC. 2013. Multiple phytohormone signals control the transcriptional response to soybean aphid infestation in susceptible and resistant soybean plants. Molecular Plant-Microbe Interactions **26**, 116–129.

Walling LL. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiology **146**, 859–866.

Xu E, Brosché M. 2014. Salicylic acid signaling inhibits apoplastic reactive oxygen species signaling. Bmc Plant Biology **14**, 155.

Yuan LB, Dai YS, Xie LJ, Yu LJ, Zhou Y, Lai YX, Yang YC, Xu L, Chen QF, Xiao S. 2017. Jasmonate regulates plant responses to postsubmergence reoxygenation through transcriptional activation of antioxidant synthesis. Plant Physiology **173**, 1864–1880.

Zhang M, Coaker G. 2017. Harnessing effector-triggered immunity for durable disease resistance. Phytopathology **107**, 912–919.